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Contribution to the morphology, biology and distribution of Citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae) and their parasitoid (Turkey)

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ABSTRACT: Contribution to the morphology, biology and distribution of Citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae) and their parasitoid (Turkey). *Misc. Pap.* 220: 1-6, 12 figs.

Effective and permanent success against to pests in biological control can only be achieved by identifying the pests, knowing the type of damage on the plants and revealing their natural enemies. In this study, the species in the citrus garden and their parasitoids were examined: *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae), *Planococcus citri* (Risso) and *Aphis spiraecola* (Patch) (Hemiptera), *Citrostichus phyllocnistoides* (Narayanan), *Cirrospilus ingenuus* (Gahan) (Hymenoptera: Eulophidae). Larvae, pupae and adults of *P. citrella* were described. The natural enemies that is effective for controlling *P. citrella* were identified and introduced. Relationship between the detected insects was evaluated.

Keywords: Citrus; Lepidoptera, Hymenoptera, Hemiptera, pest, parasitoid, Turkey.

Introduction

Citrus fruit is produced along the coasts of the Mediterranean, Black Sea and Aegean Region in Turkey. According to 2019 citrus production data, 93% of the production is supplied from the Mediterranean region. In the Mediterranean region, 90% of the production is supplied from Mersin, Adana and Muğla provinces (Anonim,2020).

Among the different insect pests of citrus crops, citrus leaf miner, *Phyllocnistis citrella* Stainton, is a serious one and damages almost all citrus cultivars (Garrido 1995; Jacas et al., 1997). *P. citrella* was originally reported from South-Eastern Asia (Clausen, 1933) and this gracillariid moth has now become established and spread rapidly throughout citrus-growing areas worldwide (Arshad et al., 2018). The distribution map of the pest (Heppner, 1993) shows that it was present in western Asian countries such as Iraq, Iran, Yemen and Saudi Arabia for several centuries, and in African countries such as Sudan and Ethiopia before 1980. In Greece it was recorded on the island of Rhodes and in Crete (Anagnou 1995, Michelakis 1997). In Turkey, the pest was recorded for the first time in

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June 1994 in the eastern Mediterranean Region (Uygun et al., 1995). It has reported that citrus leaf miner has spread to all areas where citrus is cultivated in the Mediterranean and Aegean Regions in Turkey. Güncan et al., (2009) was reported on Satsuma mandarin in Izmir province. Eşkin et al., (2016) was recorded from Ortaca (Muğla province). Koçak and Kemal (2018) recorded the specie from Adana, Antalya, Hatay and Mersin.

Much research has been done on the biological control of *P. citrella* (Argov & Rössler,1996; Orphanides, et al., 1997; Hoy, and Nguyen, 1997; Uygun et al., 1997; Kalaitzaki et al., 1999; Conti et al.,2001; Garcia-Marí et al., 2002; Garcia-Marí et al., 2004; Elekçioğu & Uygun, 2006). It should be controlled in nurseries in Turkey (Elekçioğlu, 2001). Insecticide is applied 3-5 times a year by the producers for control. This brings with it some risks in terms of environment and human health. The applied pesticides leave residues on the fruits, which is a problem especially in exported products (Eşkin et al., 2016). On the other hand, studies on the morphology of *P. citrella* are relatively limited (Rathod et al., 2020, Kernasa et al., 2008, Abo et al.,2006, Jacas and Garrido,1996). It was detected in principal studies regarding integrated strife in Turkey's citrus gardens that citrus pests have quite a high amount of natural enemies (Kansu and Uygun, 1980; Uygun, 2001). Elekçioğlu and Uygun (2013) investigated the parasitoids of *P. citrella* in grapefruit orchards in Adana, Hatay and Mersin province. This pest, which is an important issue for our country, is also included in the Agricultural Control Technical Instructions. Another important citrus pest on this list is *Planococcus citri*. It reduces fruit quality. It also causes fumagine in leaves and fruits with the sweetish substance it secretes. Carob and orange moths lay eggs in places where this pest is found. The sweet substances released by the pest become the food source of the larvae of these moths (Anonymous, 2008).

The aim of the study is to contribute to morphological information and developmental stages that will help identify the species that feed on citrus. Observations was reported and compared with previous studies. The natural enemies which is effective in controlling *P. citrella* were identified and introduced.

Materials and Methods

Samples were collected from Atayurt municipality of Silifke county in Mersin on the dates of 14-18.06.2019. Approximately 10 trees were examined, 5 contaminated leaves were collected in each tree and they were evaluated. Citrus leaves were conserved in a damp container in laboratory environment and monitored. Contaminated citrus leaves and obtained samples were photographed by Olympus stereo microscope in Gazi University of Biology Department.

Results

In the study, 5 species were identified in the citrus orchard: *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillaridae), *Planococcus citri* (Risso) and *Aphis spiraecola* (Patch) (Hemiptera), *Citrostichus phyllocnistoides* (Narayanan), *Cirrospilus ingenuus* (Gahan) (Hymenopetra, Eulophidae). Considering the number of mines per leaf, infestation of the *P.citrella* is significantly higher than the *Planococcus citri* in each host plant cistus. Two citrus pest species, *Aphis spiraecola* (Patch) and *Planococcus citri* (Risso) were detected within the pupal chamber of *Phyllocnistis citrella* (Figure 1f). *P. citri* is more intensely present under leaves and on petiole (Figure 2). A large number nymphs of *Aphis spiraecola* were observed on citrus leaf (Figure 3).

Morphology and biology of *Phyllocnistis citrella* Stainton

In the field study, abundant old, dark-colored contamination was observed on citrus leaves. *Phyllocnistis citrella* larvae were identified in all samples obtained. It was determined that moths mostly preferred light colored and new shoot leaves. Larvae (Figure 1b): It has a transparent white color, but darkens and turns yellow in the final phase. *P. citrella* larvae leave a dark mark behind them and feed on the parenchyma of the leaf. The tunnel starts in the form of a line and spreads to form circles forming a mine (Figure 1a). The dehydrated leaves curl up and the growth of the plant slows. The larvae fold down the leaves and pupate in them (Figure 1c). Pupa is yellow-brown. Patterns and colors in the form of eye spots in the prepupa become evident (Figure 1d). The growth

period of the larvae lasts about 10 days (ex pupa 28.6.2019). Adults are about 2.6 mm long. White and silvery iridescent scales can be easily seen on the front wings. At the tip of the front wing there are several black and bronze markings, as well as a black spot (Fig 1e).

Parasitoid composition of *P. citrella* Stainton

Eşkin et al. (2016) studied the parasitoid species complex of *P.citrella* in citrus groves and reported 8 Eulophidae species (*Cirrospilus brevis* Zhu, La Salle & Huang, *C. ingenuus* Gahan, *C.lyncus* Walker, *Citrostichus phyllocnistoides* Narayanan, *Diglyphus isaea* Walker, *Nechrysocharis formosa* Westwood, *Pnigalio soemius* Walker, *Semielacher petiolatus* Girault). Two parasitoids of the family Eulophidae were identified from this study in the town of Silifke in Mersin. *Cirrospilus ingenuus* Gahan and *Citrostichus phyllocnistoides* Narayan.

Cirrospilus ingenuus Gahan is an Asian parasitoid of the citrus leaf miner whose natural range includes Australia, China, India, Indonesia, Japan, Malaysia, Oman, Taiwan and Thailand (Schauff et al., 1998; Zhu et al., 2002). It was also introduced into Australia as *Cirrospilus quadristriatus*, into Cyprus and Israel as *Cirrospilus quadristriatus*, as well as Morocco, Oman, Syria, Tunisia, and Turkey (Zhu et al., 2002). This species is generally considered to be restricted in its host range to the citrus leaf miner. In this study the shiny pupa of *C. ingenuus* was founded free close to the pupal chamber. The parasitoid pupa is shiny black (Figure 4a). Adults are primarily yellow and have red eyes (Figure 4b).

Citrostichus phyllocnistoides (Narayanan) is a solitary ectoparasitoid of the late-instar larvae or of the prepupa of the citrus leaf miner (Subba & Ramamani, 1965; Bouček, 1988; Neale et al., 1995). Their pupae and adult were detected on the infected leaf epidermis in the study. The parasitoid pupa is white (Figure 5a). Adults have a large black spot on the abdomen in the form of a translucent dot (Figure 5b).

Discussion

It is thought that P. citri is the primary pest for the host in the area and creates a suitable environment for the secondary host P. citrella with the material it expels. Two species of parasitoids, *C. ingenuus* and *C. phyllocnistoides*, are associated with citrus leaf miner in the Mediterranean region of Turkey. In the Silifke county of Mersin province, which is an important region in citrus cultivation, the mass cultivation of these parasitoids and their release in the field can be used to control the *P. citrella* population more effectively. These parasitoids could be a permanent addition to the fauna of this region, making a significant contribution to the natural mortality of *P. citrella*. Therefore, breeders should be informed about the frequency and timing of application of insecticides to protect natural enemies in the field.

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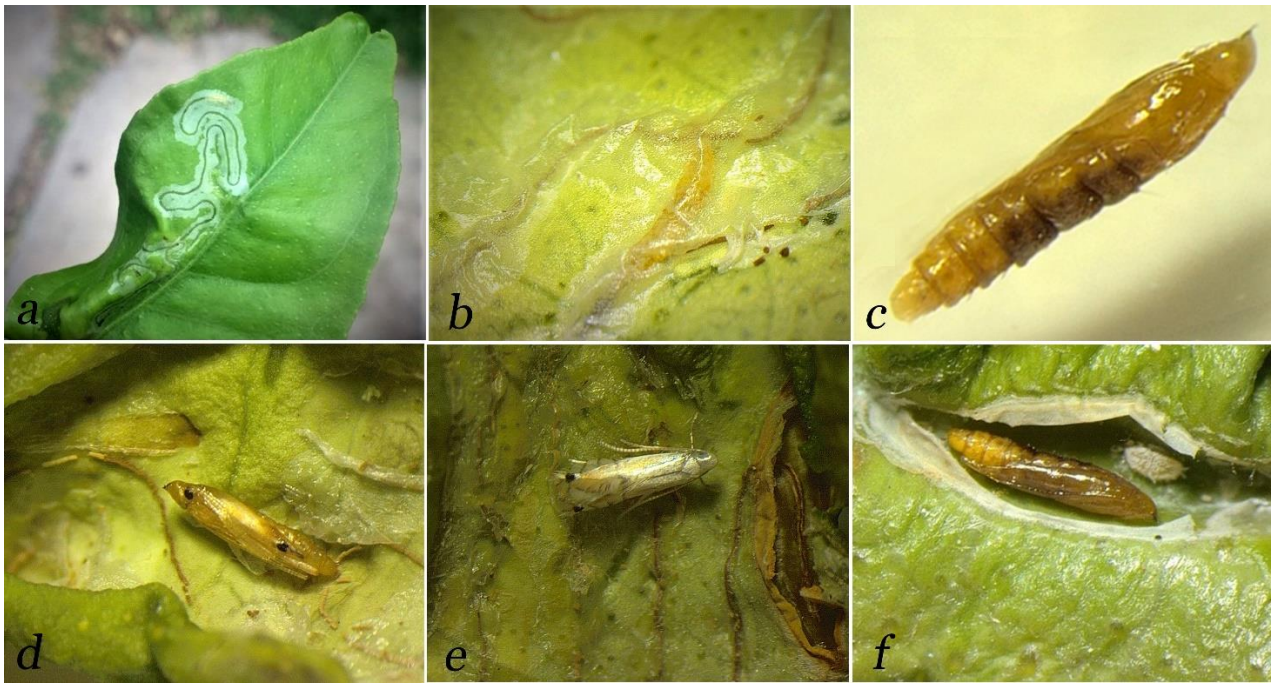

Figs. *Phyllocnistis citrella* a: A citrus leaf showing the damage caused by the feed of *P.citrella*, b: Larvae, c: Pupa, d:Preimago, e: Adult, f: Pupal chamber.

Figure 2. *Planococcus citri*.

Figure 3. The nymph of *Aphis spiraecola*.

Figs 4. *Cirropilus ingenuus* an ectoparasitoid of the citrus leafminer a: Pupa, b:Adult

Figs 5. *Citrostichus phyllocnistoides* an parasitoid of the citrus leafminer a: Pupa, b: Adult.

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A Preliminary Study on Detection DNA Barcodes of Some Pyraloidea (Lepidoptera) Species in the Van Lake Basin (Turkey)

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Abstract: A Preliminary Study on Detection DNA Barcodes of Some Pyraloidea (Lepidoptera) Species in the Van Lake Basin (Turkey). *Misc. Pap*. 220: 7-13, 11 Tables.
This article is an announcement study and detailed research is still in progress. In the study, DNA barcode information and mt COI gene characterization of Pyraloids collected from the Van Lake Basin are presented with some molecular comments.

Keywords: Barcoding, mtCOI, nucleotide positions, Pyraloidea, Turkey.

Introduction

The vast majority of known Lepidoptera species are from poorly studied groups, and it is likely that many of the species have neither been discovered nor described especially in microlepidoptera. Pyraloidea is one of the needs most research lineages of Lepidoptera due to the rich number and diversity of species it contains and the accompanying taxonomic debates. Given that pyraloids have evolved adaptations to overlapping habitats and extreme habitat environments, it is clear that the diversity they contain is unpredictable. Although genetic differentiation and phenotypic plasticity cause variations together due to wide distribution areas and similar ecological niches, it makes the diagnosis stage difficult because it causes morphological masking in these groups. For this reason, DNA barcoding and other DNA characters used in recent years give important phylogenetic signals in species delimitation or in solving higher taxonomic problems. In order for DNA-based taxonomy research to be most effective, it is necessary to obtain a large number of specimens and data from different geographies.

The Van Lake basin is one of the important centers in terms of biological diversity. The current list of the aforementioned superfamily includes 304 species from the region (Kemal and Koçak, 2018). This number constitutes 44.18% of the Pyraliodea species in Turkey. The database information under review shows that this number will increase even more. The taxa discussed in this study are preliminary for the region.

This article is an announcement about the barcode information of the Turkish Van Lake Basin Pyraloids, which form a part of the CESA collection's barcoding Project, and their future plans.

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Materials and Methods

Turkish populations of Pyraloidea were obtained from the Centre Entomological Studies Ankara (Cesa) Collection (Table 1). DNA extraction from specimens and amplification of the mtCOI gene were made according to the method of Kemal et al. (2018). Full barcodes of 24 specimens were obtained. The nucleotide positions of each barcode were calculated with the MEGA 11 program (Tamura et al. 2013). Variable and conserved sequence analyzes of *Pyrausta* and *Evergestis* populations were performed on the MathWork online portal.

Table1. Cesa registration information belong to some Turkish populations of Pyraloidea.

no	Taxon name	Collecting data	Cesa process ID	lengths of barcode
01	*Sitochroa verticalis albalis*	Van, Bahçesaray, Krapet Pass	Lep-Sit001	658bp
02	*Evergestis pazukii*	Van, Başkale, Ziyanis	Lep-Pyr003	658bp
03	*Pyrausta sp.?n.*	Van, Bahçesaray	Lep-Pyr005	658bp
04	*Evergestis sp*	Van, Bahçesaray	Lep-Pyr007	658bp
05	*Evergestis boursini*	Van, Çatak, Saklıvadi	Lep-Pyr008	658bp
06	*Pyrausta pavidalis*	Van, Bahçesaray	Lep-Pyr009	658bp
07	*Pyrausta aurata*	Van, Bahçesaray	Lep-Pyr010	658bp
08	*Epiepischnia pseudolydella*	Bitlis, Adilcevaz, Süphan Mt	Lep-Pyr011	658bp
09	*Ocrisiodes sp*	Bitlis, Adilcevaz, Süphan Mt	Lep-Pyr012	658bp
10	*Pyrausta sp*	Van, Bahçesaray	Lep-Pyr013	658bp
11	*Pyrausta sanguinalis*	Bitlis, Adilcevaz, Süphan Mt	Lep-Pyr014	658bp
12	*Pyrausta despicata*	Bitlis, Adilcevaz, Süphan Mt	Lep-Pyr015	658bp
13	*Bostra obsoletalis*	Bitlis, Adilcevaz, Süphan Mt	Lep-Pyr016	658bp
14	*Sitochroa straminalis*	Bitlis, Adilcevaz, Süphan Mt	Lep-Pyr019	658bp
15	*Phlyctaenomorpha sinuosalis*	Van, Gevaş Balaban	Lep-Pyr021	658bp
16	*Loxostege ayhanana* (paratype)	Van, Bahçesaray, Mukus Vadisi	Lep-Pyr022	658bp
17	*Evergestis boursini*	Van, Erek Mt.	Lep-Pyr023	658bp
18	*Evergestis nomadalis*	Van, Gürpınar, Başet Mt.	Lep-Pyr024	658bp
19	*Phycita imperiella*	Bitlis, Mutki, Koyunlu,	Lep-Pyr027	658bp
20	*Euzophera imperfectella*	Bitlis, Süphan, Aygır,	Lep-Pyr031	658bp
21	*Euzophera sp.*	Van, Gürpınar, Başet Mt.	Lep-Pyr032	658bp
22	*Pyrausta despicata*	Van, Bahçesaray, Mukus Vadisi	Lep-Pyr044	658bp
23	*Pyrausta aurata*	Van, Bahçesaray, Mukus Vadisi	Lep-Pyr045	658bp
24	*Pyrausta ? pellicalis*	Van, Bahçesaray, Mukus Vadisi	Lep-Pyr046	658bp

*Detailed morphological and DNA analysis studies on the species are ongoing.

Results

Due to the nature of the mtCOI gene in insects, the AT content is high in the nucleotides. According to our results, the AT ratio of first codon nucleotide changes at barcode nucleotide positions is almost above %90 in all of them. Also, the percentage of G nucleotides in the first position is more degenerate than the C nucleotides. At the second and third codon positions, AT nucleotides contained 60% on average, while GC nucleotides averaged 40%.

In *Evergestis* barcodes, the variable region is 15.2%, while the informative region ratio is 84.8%. In Pyrausta barcodes, the variable region is 24.3%, while the informative region is 75.6%. The nucleotide compositions in the DNA barcodes of the mtCOI gene have a similar distribution in 24 taxa.

Table 2. Barcode characterizations of the mtCOI gene in Turkey populations of *Evergestis* species**.**

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Başkale population of *Evergestis pazuki.*	1st	51.4	6.4	0.5	42.0	93.4	6.9
	2nd	30.6	14.2	26.5	29.0	59.6	40.7
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	32.4	15.2	14.6	37.8	70.2	29.8
Çatak population of *Evergestis boursini**	1st	45.5	6.4	2.7	45.0	90.5	9.1
	2nd	30.1	13.7	26.0	30.0	60.1	39.7
	3rd	15.1	24.7	16.9	43.0	58.1	41.6
	All	30.2	14.9	15.2	39.7	69.9	30.1
Erek population of *Evergestis boursini**	1st	48.2	7.3	0.9	44.0	92.2	8.2
	2nd	29.7	13.7	26.5	30.0	59.7	40.2
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	31.0	15.3	14.7	38.9	69.9	30.0
Gürpınar population of *Evergestis nomadalis*	1st	50.9	5.5	0.5	43.0	93.9	6.0
	2nd	30.1	14.6	26.9	28.2	58.3	41.5
	3rd	15.1	25.6	16.9	42.0	57.1	42.5
	All	32.1	15.2	14.7	38.0	70.1	29.9
Bahçesaray population of *Evergestis* sp.	1st	49.5	4.1	1.8	45.0	94.5	5.9
	2nd	30.6	12.8	26.5	30.0	60.6	39.3
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	31.8	14.0	15.0	39.2	71.0	29.0
All populations		Variable Site (%)			Informative Site (%)		
	1st	86.0			66.5		
	2nd	10.0			27.9		
	3rd	4.0			5.3		
	All	15.2			84.8		

*The genetic distance between *E.boursini* populations is over 2%. A work in progress has yet to be published.

Table 3. Barcode characterizations of the mtCOI gene in Turkey populations of *Pyrausta* species**.**

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bahçesaray population of *Pyrausta pavidalis*	1st	52.3	2.7	1.8	43.0	95.3	4.5
	2nd	31.1	14.2	26.5	28.0	59.1	40.7
	3rd	15.1	24.7	17.4	43.0	58.1	42.1
	All	32.8	13.8	15.2	38.1	70.9	29.0
Bahçesaray population of *Pyrausta aurata**	1st	44.1	5.9	3.6	46.0	90.1	9.5
	2nd	30.6	15.5	26.5	27.0	57.6	42.0
	3rd	14.6	24.7	17.8	43.0	57.6	42.5
	All	29.8	15.3	16.0	38.9	68.7	31.3
Adilcevaz Süphan Mt.of *Pyrausta sanguinalis*	1st	48.2	5.5	0.0	46.0	94.2	5.5
	2nd	31.1	16.4	26.0	26.0	57.1	42.4
	3rd	14.6	24.7	17.8	43.0	57.6	42.5
	All	31.3	15.5	14.6	38.6	69.9	30.1
Adilcevaz Süphan Mt. of *Pyrausta despicata*	1st	52.7	5.9	0.9	40.0	92.7	6.8
	2nd	31.5	13.2	26.5	29.0	60.5	39.7
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	33.1	14.7	14.7	37.4	70.5	29.4
Bahçesaray Mukus Walley of *Pyrausta despicata*	1st	53.2	5.9	0.5	40.0	93.2	6.4
	2nd	31.5	13.2	26.5	29.0	60.5	39.7
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	33.3	14.7	14.6	37.4	70.7	29.3
Bahçesaray of *Pyrausta tatarica*	1st	50.5	8.6	1.8	39.0	89.5	10.4
	2nd	31.5	14.6	25.6	28.0	59.5	40.2
	3rd	14.6	24.7	17.8	43.0	57.6	42.5
	All	32.2	16.0	15.0	36.8	69.0	31.0
Bahçesaray Mukus Walley of *Pyrausta pellicalis**	1st	50.9	3.2	0.9	45.0	95.9	4.1
	2nd	31.5	12.3	26.0	30.0	61.5	38.3
	3rd	15.5	24.7	16.9	43.0	58.5	41.6
	All	32.7	13.4	14.6	39.4	72.1	28.0
Bahçesaray of *Pyrausta* sp. n.	1st	49.5	6.8	0.9	43.0	92.5	7.7
	2nd	30.6	14.2	26.0	29.0	59.6	40.2
	3rd	15.1	24.7	17.4	43.0	58.1	42.1
	All	31.8	15.2	14.7	38.3	70.1	29.9
All populations		Variable Site (%)				Informative Site (%)	
	1st	77.5				62.9	
	2nd	20.0				18.1	
	3rd	1.8				3.6	
	All	24.3				75.6	

* Cesa process ID Lep-Pyr013 and Pyr046, Lep-Pyr010 and Pyr045 have the same barcode sequences.

Table 4. Barcode characterizations of the mtCOI gene in Turkey populations of *Sitochroa verticalis albalis* and *S.straminalis.*

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bahçesaray Karabet Pass of *Sitochroa verticalis albalis*	1st	46.4	8.2	1.4	44.0	90.4	9.6
	2nd	31.1	16.0	26.5	26.0	57.1	42.5
	3rd	15.2	24.7	17.4	43.0	58.2	42.1
	All	30.9	16.3	15.0	37.8	68.7	31.3
Bitlis Pr. Adilcevaz, Süphan Mt of *Sitochroa straminalis*	1st	45.5	5.0	1.4	48.0	93.5	6.4
	2nd	31.1	15.5	26.0	27.0	58.1	41.5
	3rd	15.1	24.7	17.4	43.0	58.1	42.1
	All	30.5	15.0	14.9	39.5	70.0	29.9

Table 5. Barcode characterizations of the mtCOI gene in Turkey population of *Loxostege ayhanana***.**

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bahçesaray, Mukus Valley of *Loxostege ayhanana*	1st	48.6	4.5	1.4	45.0	93.6	5.9
	2nd	31.1	14.2	26.0	29.0	60.1	40.2
	3rd	15.1	24.7	17.4	43.0	58.1	42.1
	All	31.6	14.4	14.9	39.1	70.7	29.3

Table 6. Barcode characterizations of the mtCOI gene in Turkey population of *Epiepischnia pseudolydella***.**

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bitlis Pr. Adilcevaz, Süphan Mt of *E. pseudolydella*	1st	49.5	4.5	0.5	45.0	94.5	5.0
	2nd	31.5	12.8	25.6	30.0	61.5	38.4
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	32.1	14.1	14.3	39.5	71.6	28.4

Table 7. Barcode characterizations of the mtCOI gene in Turkey population of *Ocrisiodes sp.*

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bitlis Pr. Adilcevaz, Süphan Mt of *Ocrisiodes sp.*	1st	48.2	3.6	0.5	48.0	96.2	4.1
	2nd	31.5	12.8	25.6	30.0	61.5	38.4
	3rd	15.1	24.7	16.9	43.0	58.1	41.6
	All	31.6	13.7	14.3	40.4	72.0	28.0

Table 8. Barcode characterizations of the mtCOI gene in Turkey population of *Bostra obsoletalis.*

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bitlis Pr. Adilcevaz, Süphan Mt of *Bostra obsoletalis*	1st	38.2	9.1	1.4	51.0	89.2	10.5
	2nd	30.6	13.7	25.6	30.0	60.6	39.3
	3rd	16.4	25.1	15.5	43.0	59.4	40.6
	All	28.4	16.0	14.1	41.5	69.9	30.1

Table 9. Barcode characterizations of the mtCOI gene in Turkey population of *Phlyctaenomorpha sinuosalis*.

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Van Pr., Gevaş Balaban of *Phlyctaenomorpha sinuosalis*	1st	30.5	14.6	9.6	45.0	75.5	24.2
	2nd	38.0	8.9	14.6	38.0	76.0	23.5
	3rd	22.6	19.4	21.9	36.0	58.6	41.3
	All	30.4	14.3	15.3	40.0	70.4	29.6

Table 10. Barcode characterizations of the mtCOI gene in Turkey population of *Phycita imperiella*.

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bitlis Prov., Mutki, Koyunlu, of *Phycita imperiella*	1st	48.2	4.5	1.4	46.0	94.2	5.9
	2nd	30.1	14.2	26.5	29.0	59.1	40.7
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	31.2	14.6	14.9	39.4	70.6	29.5

Table 11. Barcode characterizations of the mtCOI gene in Turkey populations of *Euzophera***.**

	Nucleotide Position	A (%)	C (%)	G (%)	T (%)	AT (%)	GC (%)
Bitlis Prov., Süphan, Aygırof *Euzophera imperfectella*	1st	41.1	8.2	0.5	50.0	91.1	8.7
	2nd	31.5	13.7	24.2	31.0	62.5	37.9
	3rd	16.0	25.1	16.0	43.0	59.0	41.1
	All	29.6	15.7	13.5	41.2	70.8	29.2
Van Prov., Gürpınar, Başet Mt. of *Euzophera* sp.	1st	44.1	5.0	0.9	50.0	94.1	5.9
	2nd	29.7	14.6	26.0	30.0	59.7	40.6
	3rd	15.1	25.1	16.9	43.0	58.1	42.0
	All	29.6	14.9	14.6	40.9	70.5	29.5

Discussion

In this study, DNA barcoding of 24 taxa from 5 subfamilies (Evergestinae, Odontiinae, Phycitinae, Pyralinae and Pyraustinae) of the Pyraloidea was performed. Taxa belong to 11 different areas and their collection addresses come from different ecological environments in terms of habitat characteristics and vertical distribution. The extent to which these data will differ in terms of phylogenetic systematics will be revealed by further detailed studies. Genetics research, which has become widespread in the scientific world, is still in its infancy for insects with the highest species diversity. Therefore, reliable resources to compare the obtained data are very limited.

Lepidopteran specimens in the Cesa Collection continue to be used Project Barcoding Bank of the Old World Lepidoptera (from 2018 on). CESA barcode data are the first records obtained from Turkey, even some of which are the only records in the world. Molecular taxonomic and phylogenetic studies on the evaluation of barcodes continue with hardworking.

With this first attempt on the Pyraloidea in the Van Lake Basin, the taxonomic status of the *Sitochroa verticalis albalis* subspecies from the Pyraustinae subfamily was analyzed (Kemal et al.2018). The taxonomic status of *Evergestis pazukii*, *E. boursini*, *E.nomadalis* (Evergestinae) species was evaluated for the first time by phylogenetic analyzes using barcodes (Kızıldağ, 2020b).

By obtaining the barcode of the *Loxostege ayhanana*, which was defined morphologically by Kemal and Koçak (2017), it was determined that it was a separate species in the genus by molecular analysis, and its phylogenetic position was evaluated (Kızıldağ, 2020a). The taxonomic positions of *Pyrausta* species and populations within the genus have been evaluated for the first time with such detailed phylogenetic analysis (Kızıldağ,2021). Barcodes of *Phlyctaenomorpha sinuosalis* (Odontiinae) were used for molecular taxonomic research with its taxonomical and faunistic references and distributional information (Kızıldağ and Kemal, 2019). On the other hand, the status of various taxa belonging to *Euzophera* and *Ocrisiodes* species will be investigated. Also, *Ocrisiodes* sp., *Epiepischnia pseudolydella* and *Phycita imperiella* (Phycitinae); *Euzophera* sp. for the systematic analysis of *Euzophera imperfectella* (Phycitinae) and *Bostra obsoletalis* (Pyralinae) taxa, it is necessary to reach the DNA sequences obtained from the distribution areas in Europe, Asia, the Middle East and other neighboring geographies. As a matter of fact, although there are DNA sequences made by some international research institutions, they are not available for use. In this case, it is necessary to collect samples from the distribution areas in our country and to obtain more data in order to obtain results by comparing them among Turkish populations.

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